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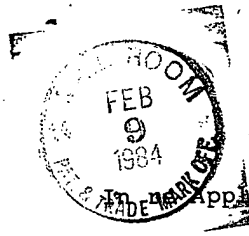

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
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RICHARD H. TULLIS	)	Examiner: Martinell
	)	
Filed: October 23, 1981	)	Group Art Unit: 174
	)	
Serial No. 314,124	)	
	)	
For: OLIGONUCLEOTIDE THERAPEUTIC AGENT AND METHODS OF MAKING SAME	)	

Los Angeles, CA 90010

February 1, 1984

DISCLOSURE STATEMENT

Hon. Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

In accordance with 37 C.F.R. 1.97, enclosed herewith, and listed on the attached PTO Form 1449, are those reference publications of which Applicant and his attorneys have become aware regarding the subject matter of the above-identified patent application. Copies of all of these references are enclosed. Included are a copy of a Patent Cooperation Treaty International Search Report and copies of the references noted therein. Three of these references have already been cited by the Examiner.

While these reference publications relate in general to various method of DNA synthesis and hybrid arrested translation, it is believed that none of them discusses, either

alone or in combination, the use of relatively small, protected oligonucleotides to specifically inhibit specific protein synthesis by hybridizing to the coding region of mRNAs.

Four of the cited references may be of more significant general interest than the remaining references. These are: Zamecnik and Stephenson, Proc. Natl. Acad. Sci. USA 75:1, 280-284 (1978); Zamecnik and Stephenson, Proc. Natl. Acad. Sci. USA 75:1, 285-288 (1978); Miller et al., Biochemistry 16:9, 1988-1996 (1977); and Ts'o et al., Jrnl. of the Am. Chem. Soc. 93:24, 6657-6665 (1971). The Zamecnik references disclose the blocking of Rous Sarcoma virus translation and replication using a thirteen base oligonucleotide. The Miller and Ts'o references disclose the increased stability of a phosphotriester protected three base oligonucleotide.

Although the four above-mentioned references are of interest, they do not affect the novelty and non-obviousness of the instant invention. The Zamecnik and Stephenson references disclose the use of an oligonucleotide to hybridize somewhere in the 5' non-coding region (terminal sequence) of RSV's mRNA. The articles themselves state that the researchers could not determine where the hybridization occurred or how translation was inhibited. The most probable hypothesis, the articles stated, was that the hybridization interfered with the circularization step of the virus.

The above distinctions between the instant invention and Zamecnik and Stephenson are crucial. The oligonucleotides in Zamecnik and Stephenson are not specific. Zamecnik and Stephenson used oligonucleotides to interfere with the replication of the Rous Sarcoma Virus by preventing

circularization. Protein synthesis does not require circularization, and therefore, their method will not function generally as a protein synthesis inhibitor. The circumstance that protein synthesis was inhibited in their experiment was fortuitous.

Further, it is most likely that the Zamecnik and Stephenson method is non-specific because it hybridizes to the 5′ non-coding region. Several different mRNA′s might have the same sequence in this region, meaning all of these mRNA′s will be blocked. In addition, the 5′ non-coding region may vary within a single type of mRNA, without a change in gene expression, meaning that the Zamecnik and Stephenson oligonucleotide might be too specific; it would not block an mRNA containing the desired gene expression, but an alternative 5′ non-coding region.

Finally, Zamecnik and Stephenson used an unprotected oligonucleotide, which would break down in vivo before having the desired effect.

Similarly, the Miller and Ts′o references do not render the present invention obvious. While they disclose the increased stability of a phosphotriester protected oligonucleotide, they deal only with a trimer (3 bases). A trimer is much too small to be specific enough to work as an effective translation inhibitor, since the probability of other mRNAs having the same three complementary bases somewhere in their base sequence is very high. A trimer would also have insufficient bonding energy to effectively inhibit translation. Further, Miller and Ts′o teach away from using a larger oligonucleotide because of synthesis problems. Under the method of synthesis used in Miller and Ts′o, the yield of phosphotriester primer was not particularly high, due to

cleavage of ester bonds in the phosphorous group during synthesis. As the number of bases in the desired oligonucleotide increases, the cleavage of these ester bonds would bring yields down to unusable levels. Thus Miller and Ts´o teach that it is not practical to use oligonucleotides much larger than three bases. This is exactly the converse of the present invention, which teaches the use of oligonucleotides containing upward of fourteen bases, preferably around 23.

It is respectfully requested that the above-identified reference publications be considered in the examination of this application and that their consideration be made of written record in the application file.

Respectfully submitted,

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Enclosures

GGK:jc